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New Tool for EDC Research

In Vivo Assay Screens for Estrogenic Effects

With more than 84,000 chemicals currently listed in the Toxic Substances Control Act inventory[1] and many of them lacking significant toxicologic data,[2] it's no easy task to pick out potential endocrine-disrupting compounds. In this issue of *EHP*, researchers describe a new *in vivo* screen they believe will improve efforts to identify high-priority chemicals for further study.[3]

High-throughput *in vitro* assays used by programs such as the U.S. Environmental Protection Agency's ToxCast™ help with preliminary screening, but they can't identify how a compound may affect the body, says first author Sylvia Hewitt of the National Institute of Environmental Health Sciences. Some *in vitro* screens simply assess whether a chemical binds to the ligand-binding domain of the estrogen receptor, but the ability to bind to the receptor says nothing about the consequences of that binding.

"It's a very one-dimensional study. Nature has engineered the uterus to be a really estrogen-responsive tissue—it's chock-full of estrogen receptors," Hewitt says. "We wanted to have a screen that goes beyond *in vitro*."

The screen uses the uterus of an ovariectomized mouse. With no circulating estrogen of its own, this model allows researchers to isolate the effects of the compound being tested. The screen consists of a panel of biomarkers and uterine responses that the authors selected as indicative of estrogenic activity. These include up- and downregulation of 50 selected genes and changes in uterine wet weight, DNA synthesis, and epithelial cell thickness and height. These biomarkers and responses are measured at 2 and 24 hours after administration of the test chemical to determine whether the substance is long- or short-acting.[3]

The researchers tested the screen on a compound known as diarylheptanoid (D3), which comes from *Curcuma comosa* Roxb., a member of the ginger family.[4] D3 is believed to have estrogenic properties[5] and is used by Thai women to relieve symptoms of menopause.[6] In the current study, both the uterine effects and the gene transcription activity of D3 at 2 and 24 hours were consistent with a short-acting estrogen.[3]

The high-throughput *in vitro* methods used in ToxCast are powerful and necessary, says Cheryl Walker, a toxicologist at the Texas A&M Health Science Center, but they do not tell us everything. "Some of the effects you will only be able to see *in vivo*, and you don't always see the same differences between two chemicals in *in vitro* assays," she explains. Walker was not involved with the new study.

Although *in vitro* screens are very useful, "it's hard to distinguish harmful and helpful, or degrees of harmful, in *in vitro* assays," says Ruthann Rudel, an environmental scientist at the Silent Spring Institute who was not involved with the study. "I think we're going to need *in vivo* methods to explore how the same estrogen receptor that binds to so many different kinds of ligands induces so many different effects."

The new screen not only enables researchers to determine whether a chemical has estrogenic effects, but also gives them the ability to classify the substance as a short- or long-acting estrogen.[3] This is important in predicting potential responses in various populations. For instance, girls and postmenopausal women may be more sensitive to effects of short-acting estrogens because their bodies do not produce long-acting estrogens.

The next big hurdle for endocrine disruptor research is figuring out how to translate the results of these and other assays into risk assessments. Rudel says that figuring out similarities and differences between different environmental estrogens is another important question to answer. Over time, she says, scientists may be able to develop classifications of estrogens that are more nuanced than simply short- or long-acting.

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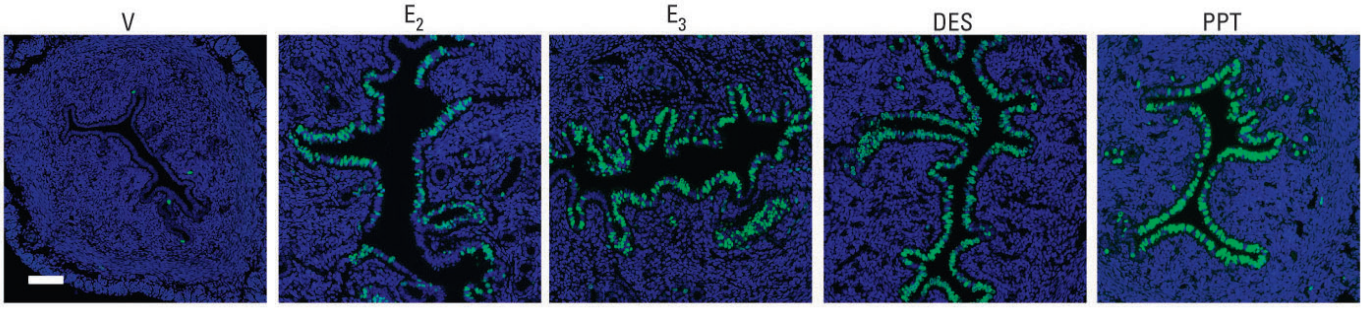

As part of developing a new *in vivo* screen for potential endocrine disruption, investigators assessed DNA synthesis in uterine tissue that was exposed to natural (E_2 and E_3) and synthetic (DES and PPT) estrogens, compared with untreated tissue (V). Their observations helped them characterize typical responses to estrogenic compounds, which may be useful in identifying chemicals with estrogenic activity.

Image: Hewitt et al. (2015)[3]